Conclusion: Prostaglandin E2, the predominant eicosanoid produced by OA chondrocytes, exerts catabolic effects within cartilage. These deleterious effects require signaling via the EP4 receptor, which should be considered as a potential target for disease modifying agents in OA.

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MODULATION OF LUBRICIN GLYCOSYLATION AND MULTIMERIZATION IN SYNOVIAL LINING CELLS BY TGF-b AND LEUKEMIA INHIBITORY FACTOR

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Study Aims: We report here the existence of a novel posttranslational processing pathway for lubricin in synovial lining cells, and show opposed regulation of glycosylation and aggregation by growth factors (TGFb) and cytokines (IL6 and LIF) involved in post-injury joint disease.
Histological and cellular changes to the synovial lining are frequently reported in both animal models and human OA. While synovial histopathology is well described, very little is known about the pathway that links cellular changes in this tissue to degradation and remodeling of joint tissues which is a hallmark of late stage OA. A major function of the synovial lining cell is the secretion of macromolecular components of the joint fluid, such as hyaluronan (HA) and lubricin (LB). We report here novel observations on the effect of OA-relevant cytokines on the biosynthesis and assembly of lubricin multimers.
Methods: Synovial membranes from bovine metacarpalphalangeal joints, were digested in collagenaseP (0.1%). Tissue remnants were removed with a cell sieve, cells washed and plated at 1x106 cells/100 mm dish in DMEM/20% FCS. Following 1 passage, confluent cells were supplemented for 48 h with and without TGFb alone and in combination with LIF. Conditioned media fractionated by MonoQ ion exchange and separated isoforms of lubricin were further analyzed by Agarose GE and Westerns with anti-peptide antibodies (JSCIQQ and JSCLPN) to the N-terminal (19-IQQVSSQ DLSC) and the C-terminal (1345-LPNVVTSAISLPNIRKPD) sequences.
Results: AGE analyses of native and reduced/alkylated preparations showed that control cultures secreted lubricin as both, monomers and disulfide-bonded aggregates. TGF-b caused a marked increase in the production of lubricin, predominantly as a monomeric low-charge glycoform much of which lacked the N-terminal epitope IQQ. When cultures were exposed to LIF, a 'shift' back to the 'control' culture condition occurred with synthesis of the low charge glycoform reduced, and less N-terminal epitope loss. To examine the effect of LIF on lubricin organization in the cell-associated matrix, cytokine-treated cell layers were fixed in Histochoice and examined by confocal IHC. The control cultures showed intense staining of the ECM domain closely associated with the cells. Treatment with TGF-b resulted in a marked reorganization of LB staining with very few intense ECM-positive cells remaining, most cells showed only a diffuse staining which appeared to be intracellular. When LIF was added at the same time as TGF-b the LB-positive cells now displayed the intense cell-associated ECM typical of control untreated cells, supporting the idea that LIF can restore the 'mature secretory' synovial phenotype in a post-injury joint. An investigation of the signaling pathway for this LIF-mediated effect suggests that it involves an intracellular response of SOCS1.

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PROTECTIVE ROLE OF HEME OXYGENASE-1 IN HUMAN OSTEOARTHRITIC CHONDROCYTES

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Aim of Study: Heme oxygenase-1 (HO-1) is induced as an adaptive mechanism against tissue injury. We have recently demonstrated that basal expression of HO-1 in human osteoarthritis (OA) chondrocytes is negatively regulated by inflammatory cytokines. In this study we have investigated whether the modulation of the HO-1 pathway could be able to attenuate the effects of OA.
Methods: We have treated human OA chondrocytes with a HO-1 inducer, cobalt protoporphyrin IX (CoPP), or with a CO donor, tricarbonyldichlororuthenium(II) (TCCT) and assessed different OA markers in chondrocytes stimulated with IL-1β. Cartilage specimens were obtained from 17 patients with diagnosis of advanced OA undergoing total knee joint replacement. Chondrocytes were used in primary culture. Cells were treated with 100 U/ml IL-1β, and 10 μM CoPP or 100 μM TCCT for 24 h.
Results: CoPP, and interestingly TCCT, significantly increased HO-1expression in non-treated and IL-1β stimulated chondrocytes versus basal chondrocytes. Both drugs decreased the production of different inflammatory mediators (IL-6, TNFα, NO, PGE_2), and IL-10. We have evaluated some markers of cell cycle arrest, apoptosis induction, and phenotype changes. TCCT was able to down-regulate p21 and Bax protein expression. Treatment with this agent resulted in a lower number of cells with DNA fragmentation and absence of droplets in cytoplasm in comparison with control cells stimulated with IL-1β. In studies of cartilage degradation, CoPP was able to reduce proteoglycan degradation in OA explants, whereas TCCT down-regulated IGFBP-3 expression in chondrocytes stimulated with IL-1β, which may be related to a lower catabolism of cartilage matrix molecules.
Conclusions: These results suggest a correlation between HO-1and the reduction of tissue damage in human OA chondrocytes and support a protective role for HO-1 in OA.

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BASIC-FGF INHIBITS THE ANABOLIC ACTIVITY OF IGF-I AND OP-1 IN HUMAN ARTICULAR CHONDROCYTES

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Aim: IGF-I and OP-1 are key anabolic factors in adult articular cartilage. Recent studies have shown that bFGF is released from the adult cartilage matrix upon cartilage injury and mechanical stimulation. The aim of this study was to determine the effects of bFGF on chondrocyte proliferation and matrix synthesis in the presence of IGF-I and OP-1.
Methods: Human articular chondrocytes were isolated from normal ankle cartilage obtained from 9 tissue donors with ages of 47-73 years. Cells were cultured in alginate beads or as cartilage explants in serum-free media supplemented with mini-ITS with or without IGF-1 (100ng/ml), OP-1 (100ng/ml) or bFGF (0-100ng/ml). The cells and their pericellular matrix were visualized with the particle exclusion assay. Cell survival (LIVE-DEAD Cell Assay), proliferation (DNA levels), proteoglycan synthesis (sulfate incorporation), and total proteoglycan accumulation (DMB dye binding assay) were measured after 21 days of culture in algi-

nate beads. Sulfate incorporation was measured over the final 4 hours of culture at day 10 or 21 day for explants.
Results: bFGF added to any of the culture conditions did not alter cell survival. bFGF in alginate cultures resulted in the formation of cell clusters that lacked a visible pericellular matrix and the addition of bFGF to IGF+OP-1 treated cultures was seen to inhibit the ability of the latter growth factors to stimulate pericellular matrix formation. Chondrocyte proliferation was stimulated at doses above 1ng/ml of bFGF with a maximal 2-fold increase at100 ng/ml. However, the 1ng/ml dose of bFGF was sufficient to stimulate proliferation when combined with IGF-I or with IGF+OP-1, the latter resulting in the greatest stimulation of proliferation which was over 3.5-fold of control. The increase in cell numbers correlated with a decrease in total proteoglycan (PG) levels accumulated per cell (DMB/DNA) which was 40% of control at 100ng/ml bFGF. Although IGF-I, OP-1, or IGF-I+OP-1 significantly increased proteoglycan levels above controls, the addition of bFGF to these cultures resulted in significant inhibition of proteoglycan synthesis below levels of untreated control (45% of control with IGF-I+bFGF vs 128% of control with IGF-I alone; 55% of control with OP-1+bFGF vs 186% of control with OP-1 alone and 40% of control for bFGF+IGF-I+OP-1 vs 165% of control with IGF-I+OP-1). Likewise, sulfate incorporation (as a measure of new PG synthesis) in response to IGF-I and OP-1 alone or together was completely inhibited by 50ng/ml bFGF. Similar effects on proteoglycan synthesis were obtained in cartilage explants where 50ng/ml bFGF totally inhibited the ability of IGF-I or OP-1, alone or together, to stimulate sulfate incorporation.
Conclusion: In adult human articular cartilage, bFGF stimulates proliferation at doses above 1ng/ml and significantly inhibits the anabolic activity of IGF-I and OP-1. The results suggest that excessive release of bFGF from the cartilage matrix during injury, with loading, or in arthritis could contribute to reduced anabolic activity in cartilage.

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EXPRESSION AND SUBCELLULAR LOCALIZATION OF COX 1 AND 2 AND THEIR ASSOCIATED TERMINAL SYNTHASES, cPGES AND mPGES, IN IL-1-STIMULATED CHONDROCYTES

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Purpose: Expression of PGE_2 depends upon simultaneous expression of a cyclooxygenase (COX-1, or COX-2) and an associated terminal PGE synthase (PGES), in order to convert the COX product PGH_2 into PGE_2. We examined the expression and subcellular localization of COX-1, COX-2, microsomal PGES (mPGES, inducible, presumed to associate with COX-2) and cytosolic PGES (cPGES, presumed to associate with COX-1), in chondrocytes (C28I2 cell line kindly provided by Mary Goldring) stimulated with IL-1.
Methods: C28I2 cells were stimulated (various times) with IL-1beta (10ng/ml). Lysates were analyzed for COX-1, COX-2, cPGES and mPGES protein and mRNA. Cells fractionated into nuclear and cytoplasmic components were analyzed for COXs and PGESs by western blot. Supernatants were analyzed for PGE_2.
Results: mRNA and protein for both COX-1 and cPGES were present in unstimulated C28I2 chondrocytes; COX-1 and cPGES mRNA and protein levels did not change substantially with IL-1 stimulation over a period of 24 h. In contrast, mRNA and protein for both COX-2 and mPGES were not expressed in unstimulated C28I2 cells, but were upregulated by IL-1. COX-2 protein was observed as early as 4 h after stimulation, reaching maximal levels shortly thereafter. mPGES protein expression was observed somewhat later, with a peak at 24 h. Subcellular fractionation revealed that COX-1 was localized primarily to the nucleus (nuclear membrane), with unchanged distribution in response to IL-1. COX-2 and mPGES also were localized to the nucleus, but only after IL-1-induced expression. In contrast, cPGES was localized primarily to the cytosol, with little or no cPGES in the nuclear fraction of unstimulated cells. Upon IL-1 stimulation, the amount of cPGES at the nuclear membrane rapidly and transiently increased (maximum 236% of unstimulated at 30 min, return to baseline by 4 h), indicating early translocation of a portion of cPGES to the nucleus, and suggesting a possible COX-1/mPGES assemblage. Measurement of extracellular PGE_2 in response to IL-1 revealed three phases: an early accumulation (peak 30 min; 146% of unstimulated control) presumably related to assembly of the COX-1/cPGES system, a plateau phase (126% of control at 2 h), and a late phase of production (1645% of control at 24 h), presumably related to a COX-2/mPGES system.
Conclusion: Our data suggest that, in response to IL-1, PGE_2 production may occur through sequential generation and/or assembly of two sets of PGE_2-producing systems: an early system composed of constitutively expressed COX-1/cPGES, requiring translocation of cPGES to the nuclear membrane where a PGE synthetic complex may be assembled, and a later, COX-2/mPGES system that depends on stimulated, nuclear membrane expression of both of these proteins.

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LOSS OF ESTROGEN LEADS TO CARTILAGE DEGRADATION

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Purpose: Cartilage degradation is a hallmark in osteoarthritis (OA) and loss of estrogen has been shown to be associated with increased prevalence of OA in postmenopausal women. It remains to be elucidated whether this potential chondroprotective effect of estrogen is mediated directly or indirectly on chondrocytes.
We investigated i) the effect of estrogen treatment or prevention on cartilage health in the rat OVX model and ii) the direct effects of estrogen on cartilage turnover in an cartilage explant model.
Materials and methods: *In vivo*, forty 7-month old Sprague-Dawley rats were divided into four groups. One group was subjected to sham operation, whereas the others to OVX, followed by treatment with either vehicle alone, 17-b-estradiol [0.25mg/pellet s.c.] initiated instantly or 3 weeks after surgery and continued until termination after 9 weeks. Cartilage erosion was evaluated histologically by assessment of proteoglycan staining followed by microscopic measurement of eroded surface length in the articular cartilage. *Ex vivo,* for articular cartilage explants culture, 14±2 mg cartilage was isolated from bovine heifer stifle joints, and cultured in 96-well plates. Cartilage degradation was induced by oncostatin M (OSM) [10 ng/mL] and TNF-α [20 ng/mL], in the presence or absence of 17-β-estradiol [0.1 nM – 1 μM]. In both the in vivo and ex vivo settings, cartilage degradation was estimated by measuring the concentration of the C-telopeptide fragments of collagen type II (CTX-II) in serum and conditioned medium, respectively.
Results: Ovariectomy induced a 66% increase in serum CTX-II levels compared with sham-operated animals ($P<0.001$), indicating an increase in cartilage degradation. In accordance with the accelerated collagen degradation, there was a 53% increase in the extent of cartilage erosion compared to sham-operated